DEVELOPMENT OF INTEGRATED SYSTEM FOR BIOMIMETIC CO_2 SEQUESTRATION USING THE ENZYME CARBONIC ANHYDRASE

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INTRODUCTION

Many possible approaches to carbon sequestration are being investigated by researchers worldwide (see, e.g., Bond et al., 1999 a, b; Department of Energy, 1997; Hendriks, 1994; Herzog, 1997; Knotek and Eisenberger, 1998; Lackner et al., 1995; Yamada, 1998). This is good, because the scale of anthropogenic CO_2 emissions is so huge that sequestration of anything more than a small fraction of it is likely to require a combination of different approaches. Most sequestration studies have been based on the assumption that CO_2 would first have to be separated from the remainder of the exhaust gases from fossil-fuel combustion. It could then be disposed of, for example, in DOG (depleted oil and gas) wells, in deep saline aquifers, in the deep ocean, or through deposition into minerals such as peridotites or serpentinites. A common theme for all of these approaches is that they involve the need to concentrate and, for the most part, transport CO_2. However, whilst it is technically feasible to remove CO_2 from flue gases with existing technology (Hendriks, 1994; Yamada, 1998), the removal systems require large amounts of capital and energy, and could raise the cost of busbar electricity, for example, by 50% (Department of Energy, 1997). Transportation of CO_2 by pipeline as a supercritical fluid is a well-established technology in EOR (enhanced oil recovery), but still is not cheap.

Our present research is aimed at the development of a novel biomimetic approach to CO_2 sequestration. The intent is to develop a CO_2 scrubber that can be used to reduce CO_2 emissions from, for example, fossil-fuel-burning power plants, based on the use of an enzyme or biological catalyst. The resulting sequestration system would offer several potential advantages, including: a plant-by-plant solution to emission reduction; no costly CO_2 concentration and transportation steps; a safe, stable, environmentally benign product; and an environmentally benign process. Proof of principle has already been demonstrated (Bond et al., 1999 a, b). The present emphasis will be on the performance of the enzyme in the presence of other chemical species likely to be present in the industrial situation. It is useful first, however, to summarize the biomimetic approach, in order to put the present results in context.

Atmospheric levels of CO_2 are much lower today than they were early in the earth's history. Carbonate minerals, such as calcite, aragonite, dolomite, and dolomitic limestone, comprise a massive CO_2 reservoir, estimated (Wright et al., 1995) to contain an amount of carbon equivalent to $150{,}000 \times 10^{12}$ metric tons of CO_2. Thus carbonate minerals offer a geologically proven, safe, long-term repository for CO_2. If anthropogenic CO_2 can be fixed into solid carbonate form, such as calcium carbonate, then we have a stable and environmentally friendly product. The problem, of course, is one of rate.

In order to address the problem of rate, we adopted a biomimetic approach (Bond et al., 1999 a, b). It is useful to keep in mind here that we are defining a biomimetic approach as one in which a particular aspect of a biological process or structure is identified and applied to solve a specific non-biological problem (Bond et al., 1999 a). In other words, it is an approach in which:

- We have a specific engineering problem to solve.
- We identify a biological system in which an analogous engineering problem has been solved.
- We use the enabling part of that system, whether it be a structural design, a processing route, or a biochemical component, to solve our engineering problem.

In the present instance, we examined the rate-limiting step in the chemistry of CO_2 fixation into calcium carbonate in aqueous solution, and then considered what lessons could be learned from biological systems in order to accelerate that step. Calcium carbonate precipitates readily from aqueous solution given a suitable saturation of calcium and carbonate ions, and so the issue becomes one of how to produce carbonate ions rapidly from CO_2 and H_2O (Wilbur and Simkiss, 1968). One important parameter to be considered is pH, because of its strong effect on the proportions of the carbonic species present (Loewenthal and Marais, 1978), and because, at low pH, carbonates will tend to dissolve rather than precipitate. Although carbonate could be formed rapidly at high pH, this would pose both economic and environmental concerns, and hence a

process that operates at very mildly basic pH values would be desirable. Gaseous CO_2 dissolves rapidly in water, producing a loosely hydrated aqueous form (Quinn and Jones, 1936; Keene, 1993). Three additional reactions are then required to form carbonate ions at moderate pH:

- Hydration of aqueous CO_2 to produce carbonic acid (H_2CO_3).
- Dissociation of carbonic acid into bicarbonate ions and protons.
- Production of carbonate ions from bicarbonate ions.

By far the slowest of these reactions is the hydration of CO_2 (Keene, 1993).

A solution to the problem of accelerated CO_2 hydration, in fact, already exists in biological systems. The carbonic anhydrases (CAs) are a broad group of zinc metalloenzymes that are ubiquitous in nature (Brown, 1990; Dodgson et al., 1991; Pocker, 1990). They are among the fastest enzymes known, and they catalyze the reversible hydration of CO_2. The fastest CA isozyme known is the human isozyme HCA II, each molecule of which can hydrate at least 1.4×10^6 molecules of CO_2 per second (Khalifah and Silverman, 1991); the catalyzed hydration occurs at or near the diffusion-controlled limit for the encounter rate of enzyme and CO_2. Thus, if we use CA to catalyze the hydration of CO_2, it should be possible to fix large quantities of CO_2 into carbonate form, without recourse to caustic conditions. [Another group has, in fact, been looking at CA as a catalyst for short-term aqueous sequestration of CO_2 for use in completely closed systems, such as a space station (M. Trachtenberg, personal communication).]

Feasibility of our biomimetic approach was demonstrated (Bond et al., 1999 a, b), based on two types of experiment. One was designed to show acceleration of the overall process of forming a solid product (calcium carbonate) in the presence of CA. This involves a series of steps beyond the hydration of CO_2, but is vital to show potential industrial applicability. The other was designed to demonstrate the accelerated hydration of CO_2 in the presence of CA. This shows catalysis of a single reaction, and hence is applicable also to comparisons of enzyme performance for different isozymes, and under different conditions. Following the successful proof of principle, several topics were identified as needing further study, and some of these are discussed in Bond et al. (1999 a, b). One of these topics is optimization of the catalyst. The activity and lifetime of the enzyme will be influenced by a range of factors, including pH, temperature, and other ions present. The ions present will depend on the source of the water used (seawater or freshwater), as well as on the other species present in the flue gases.

Some inhibition of CA activity by various anions has been reported previously (see, e.g., Maren et al., 1976 [delta pH]; Pocker and Stone, 1967 [*p*-NPA assay]). By the far the most potent of the inorganic anionic inhibitors of CA, however, is CN^-, which is not an issue for the proposed application. Very small amounts (unlikely to exceed 100 ppm) of SO_x (Electric Power Research Institute, 1984) and NO_x may be present in the flue gases. Higher concentrations of anions are likely in the water used, particularly, of course, if it is seawater.

EXPERIMENTAL

A suitable catalyst (or isozyme) for an industrial-scale CO_2 scrubber will have to be fast, robust, and capable of being produced in large amounts cost effectively. The simplest and cheapest means of obtaining large amounts of CA will be by means of overexpression by a genetically modified bacterial system, and we are working with two different isozymes for which other research groups have successfully cloned genes. In the short term, however, initial experiments on anionic inhibition have been performed on BCA (bovine erythrocyte CA), purchased in purified form from Sigma Chemical Corporation.

The method that has been used to show the accelerated hydration of CO_2 is a delta pH method (Henry, 1991). CA catalyzes the reversible hydration reaction between CO_2 and H_2O, producing HCO_3^- and H^+. This production of protons leads to a change in pH as the reaction proceeds towards equilibrium. Measurement of this pH change as a function of time forms the basis of the delta pH method. Measurements are usually made at temperatures in the range 0-5°C, to slow the enzyme-catalyzed reaction which is otherwise so rapid that initial rates are hard to measure (Henry, 1991). A World Precision Instruments Bee-Trode pH electrode and Dri-Ref system, with an ATC (automatic temperature compensation) probe, connected to an Orion Sensorlink pH data acquisition system, was used for temperature-compensated pH monitoring. Activities were measured and compared for 30μg/ml BCA in 2.5 mM aqueous CO_2 solution, in the presence of different concentrations of Na_2SO_4 and $NaNO_3$, in 25mM pH 7.4 tris buffer, at 1-3 °C. CA activity in ASW-based solution (artificial seawater) was also compared to that in deionized-water-based solution. ASW was produced by dissolution of Sigma artificial-seawater salts, 38g of which were added to 1 liter of deionized water prior to bubbling with CO_2. In this case, the weak buffering was achieved with Barbital buffer.

A *p*-NPA assay has also been used to monitor the activity of CA, based on the enzyme-mediated hydrolysis of para-nitrophenyl acetate (Pocker and Stone, 1967). The same enzyme active site that is responsible for acceleration of CO_2 hydration also accelerates this hydrolysis reaction, which yields a bright yellow product that absorbs at 405 nm and can be determined spectrophotometrically. Due to stearic factors, however, the reaction rate is much slower for the hydrolysis reaction than for the hydration of CO_2. Thus the enzyme-accelerated hydrolysis rates can be conveniently monitored without cooling (in contrast to the delta pH measurements of accelerated CO_2 hydration), making the *p*-NPA assay useful for a first look at the influence of different parameters on CA activity. Activities were measured and compared for 20μg/ml BCA in the presence of different concentrations of Na_2SO_4 and $NaNO_3$, in 50mM pH 7.4 tris buffer, at 25°C. Stock solutions of 0.0025g/ml *p*-NPA were prepared in acetonitrile, which was used to prevent spontaneous decomposition of *p*-NPA in air or in water. 10% (v/v) was added into each sample solution. The absorbance intensity at 405nm for the yellow product, *p*-nitrophenol, was followed versus time with a Hitachi-330 spectrophotometer. The slope of absorbance versus time was defined as the activity gradient.

RESULTS

Absorbance-versus-time plots from the *p*-NPA assay are shown for SO_4^{2-} concentrations ranging from 0.5 mM up to 200 mM in Figure 1. There is little inhibition at concentrations up to 5 mM (480 ppm), but there is significant inhibition by 50 mM. Similar plots are shown for NO_3^-, again over a concentration range of 0.5 mM up to 200 mM, in Figure 2. Again, large-scale inhibition starts somewhere between 5 mM (310 ppm) and 50 mM.

Delta pH data for different SO_4^{2-} and NO_3^- concentrations, ranging from 5 mM to 200 mM, are shown in Figures 3 and 4 respectively. There is little indication of inhibition at concentrations below 100-200 mM. Figure 5 shows a comparison of enzyme activity in ASW-based solution versus DI-water-based solution. The enzyme is seen to perform well in both solutions.

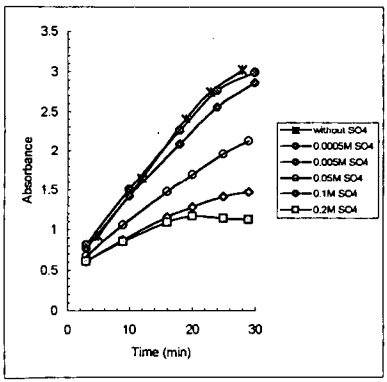

Figure 1. *p*-NPA activity of BCA in various concentrations of SOx, in tris buffer.

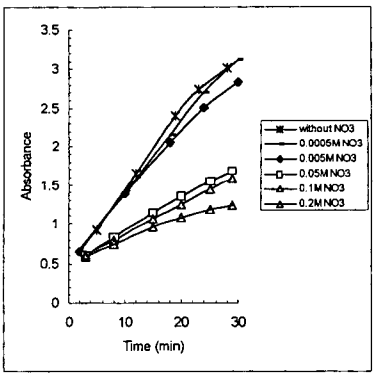

Figure 2. *p*-NPA activity of BCA in various concentrations of NOx, in tris buffer.

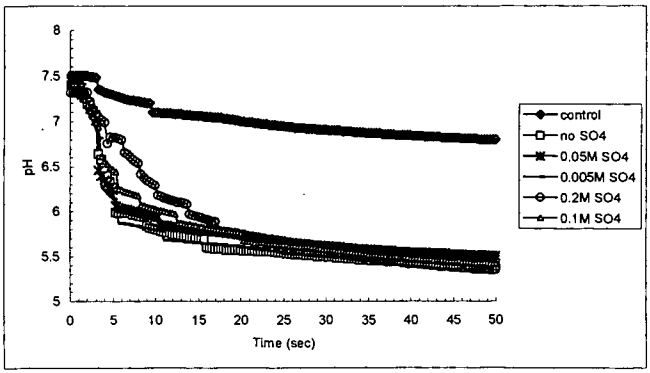

Figure 3. Delta-pH activity of BCA in various concentrations of SOx, in tris buffer.

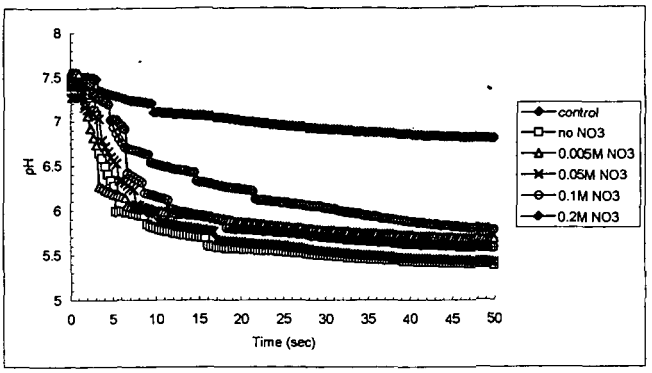

Figure 4. *Delta-pH activity of BCA in various concentrations of NOx, in tris buffer.*

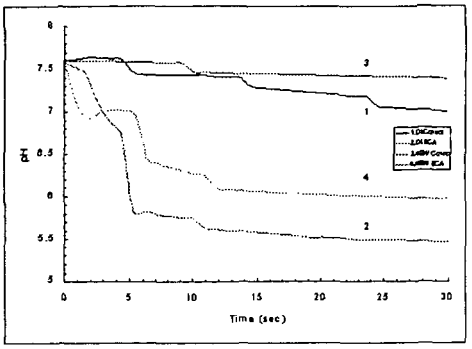

Figure 5. Delta-pH activity of BCA in artificial seawater (ASW) and de-ionized water (DI) tested in barbital buffer.

DISCUSSION

It is interesting to note that the enzyme appears to be less susceptible to inhibition as determined by the delta pH technique, than by the *p*-NPA assay. It should be remembered, however, that the enzyme is also a much less efficient catalyst for the hydrolysis of *p*-NPA than it is for the hydration of CO_2, and hence it is perhaps not surprising that that already somewhat difficult catalytic action should be more easily inhibited. It is clear that, for the present purposes of catalyst optimization, the *p*-NPA assay has a useful role to play for convenient initial screening, but the final assay should be delta pH.

Given the low levels of SO_x and NO_x that would be present in flue gases reaching a CO_2 scrubber located behind a sulfur scrubber, it appears very unlikely that either of these species would present an inhibition problem to the enzyme in such a system. It also appears unlikely that the type of water used will pose an inhibition problem, even for seawater, as represented here by the ASW-based solution. Similar experiments will be performed on the other isozymes, produced by bacterial overexpression, that we are currently investigating.

CONCLUSIONS

The enzyme, carbonic anhydrase, is the biological catalyst responsible for the interconversion of CO_2 and bicarbonate in living organisms. The present research is aimed at the development of a CO_2 scrubber that can be used to reduce CO_2 emissions from, for example, fossil-fuel-burning power plants. In this system, the enzyme works as a catalyst to accelerate the rate of CO_2 hydration for subsequent fixation into stable mineral carbonates, the counterions for which may be supplied from such sources as waste brines from desalination operations. Proof of principle has already been demonstrated. One of the requirements for the enzyme will be that it must be able to function in the presence of other chemical species likely to be present in the industrial application. The present results show excellent enzyme activity in the presence of low levels of SO_x and NO_x (that might be expected from flue gases) and also in solution representative of seawater.

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